

CLAIMS

1. A method of producing a *thy A* strain of *Vibrio cholerae* comprising the 5 step of site-directed mutagenesis in the *V. cholerae* chromosome for the deletion and/or insertion of gene nucleotides at the locus of the *thy A* gene having essentially the nucleotide sequence SEQ ID NO: 1 of FIG. 1.

2. A *Vibrio cholerae* *thy A* strain which is a Δ *thy A* strain lacking the functionality of the *thy A* gene.

10 3. A Δ *thy A* strain of *Vibrio cholerae* according to claim 2 comprising one or several episomal autonomously replicating DNA elements having a functional *thy A* gene that enables the strain to grow in the absence of thymine in the growth medium.

4. A Δ *thy A* strain of *Vibrio cholerae* according to claim 3, wherein the episomal autonomously replicating DNA element is a plasmid.

15 5. A Δ *thy A* strain of *Vibrio cholerae* according to claim 3 or 4 comprising a foreign *thy A* gene.

6. A Δ *thy A* strain of *Vibrio cholerae* according to claim 5, wherein the foreign *thy A* gene is an *E. coli* gene.

7. A Δ *thy A* strain of *Vibrio cholerae* according to any one of claims 3 to 20 6, wherein the one or several episomal autonomously replicating DNA elements also comprise a structural gene encoding a homologous or heterologous protein.

8. A Δ *thy A* strain of *Vibrio cholerae* according to claim 7, wherein the encoded protein is selected from heat labile enterotoxin B-subunit of *Escherichia coli* (LTB) and *Schistosoma japonicum* glutathione S-transferase 26 kD protein (GST 26 25 kD).

9. A nucleotide sequence of a *thy A* gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 1 of FIG. 1.

10. A nucleotide sequence of a 5'- flanking region of a structural *thy A* gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 2 of FIG. 2.

30 11. A nucleotide sequence of a 3'- flanking region of a structural *thy A* gene of *Vibrio cholerae* having essentially the nucleotide sequence SEQ ID NO: 3 of FIG. 3.

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12. A protein encoded by a nucleotide sequence of a *thy A* gene of *Vibrio cholerae* according to claim 9.

13. A protein according to claim 12, wherein the protein has the amino-acid sequence SEQ ID NO: 4 of FIG. 4.

5 14. A protein encoded by a nucleotide sequence of a 5'- flanking region of a structural *thy A* gene of *Vibrio cholerae* according to claim 10.

15. A protein according to claim 14, wherein the protein has the amino-acid sequence SEQ ID NO: 5 of FIG. 5.

16. A vaccine comprising as an immunising component a *Vibrio cholerae* 10 Δ *thy A* strain according to any one of the claims 2 - 8 or a *thy A'* strain of *Vibrio cholerae* produced by the method of claim 1.